CHAPTER I

INTRODUCTION

PART I

ASCORBIC ACID LOCALIZATION AND METABOLISM IN ALBINO RATS

Ascorbic acid is an important biologically active reductant that plays a dynamic role in several oxidoreduction reactions in animal tissues via the formation of its free radical, monodehydroascorbic acid and charge transfer complex formation with macromolecules. Its role in enzyme activation, biosynthetic processes, steroidogenesis, metabolism of spermatozoa and other tissues, drug detoxification mechanisms, growth and regenerative processes and in stress mechanisms as a source of electron energy is well known (Chinoy, 1969 a, 1970 a, b; 1971 a, b; 1972 a, b; 1973; Chinoy et al., 1974 a, b; Chinoy and Parmar, 1975 a, b; Chinoy and Sheth, 1976; 1977 a to d; Chinoy and Buch, 1977 a, b; Chinoy et al., 1977; 1978 a to e; Chinoy and Sanjeevan, 1978 a; Chinoy, 1978; Seethalakshmi and Chinoy, 1978; Sebrell and
Harris, 1967; Lewin, 1976; Datta and Sanyal, 1978). The extensive biochemical and electron spin resonance spectrometric studies carried out in our laboratory on the metabolic turnover pattern of ascorbic acid in numerous animal tissues under various altered physiological conditions have revealed for the first time that the animal tissue metabolism is energized not only by the high energy phosphate (~P ) but also directly by the paramagnetic electron flow from monodehydroascorbic acid which functions as an electron donor. Therefore, it was thought worthwhile to study the localization pattern of ascorbic acid in the testis and epididymis as well as the turnover pattern of ascorbic acid in different components of the testis and epididymis of albino rats.

PART II
STUDIES ON MALE CONTRACEPTIVE TECHNIQUES AND THEIR EFFECTS ON MALE REPRODUCTIVE FUNCTIONS

1. VASECTOMY

Vasectomy is one of the most widely used contraceptive methods in male, specially in India, since it involves a simple operative procedure and no hospitalization. However, there are conflicting reports regarding its
effects on the male reproductive system. Studies on rodents, dogs, bulls and human beings have revealed that vasectomy has no adverse effects on pituitary weight, gonadotropin activity, histology of genital organs, spermatogenesis, sperm metabolism, androgenicity of the testis, secretory functions of accessory reproductive organs and fertility (Kar et al., 1965; Flickinger, 1972, 1973, 1975; Kwarts and Coffey, 1973; Easterday et al., 1973; Wieland et al., 1972; Lohia and Dixit, 1974; Johnsonbaugh et al., 1975; Neaves, 1974; 1975; Meenakshi et al., 1976 a, b; Bedford, 1976; Sheth, 1976; Buch, 1976; Chinoy et al., 1978 a,b). Recent studies from our laboratory have also revealed that the circulating levels of testosterone in vasectomized volunteers were not altered in comparison to those of normal volunteers (Chinoy et al., unpublished observations). Similarly, the spin concentrations of manganese free radical were not significantly different in the cauda epididymal sperm suspension of normal and vasectomized rats, showing thereby that vasectomy does not affect the utilization of manganese by epididymis (Chinoy and Seethalakshmi, 1978 c). On the contrary, several workers have shown a decline in organ weights,
transitory degenerative changes in testis, structural changes in spermatozoa and epididymis, disturbances in spermatogenesis, reduced sperm counts, a decline in androgen levels concomitant with the reduction in the amount of urinary 17-ketosteroids and the formation of spermatic granuloma in the epididymis / vas deferens at the site of ligation (Laumas and Uniyal, 1967; Kubota, 1969; Igoboeli and Rakha, 1970; MacMillan et al., 1968; Alexander, 1972, 1973; Sacher and Schilling, 1973; Vare and Bansal, 1973; Bruschke et al., 1974; Deerick et al., 1974; Kinson and Layberry, 1975; Chatterjee et al., 1976). Significant quantitative changes in some of the contents of human seminal plasma in vasectomized males have also been reported (Nun et al., 1972; Gregoire and Moran, 1973; Brummer, 1973). Procedural variations, species difference, age of the animal and duration of the operation have been suggested as responsible for the differences in the results obtained (Flickinger, 1972), whereas Neaves (1974) suggested that factors other than procedural differences were responsible for the controversial data. Meenakshi et al., (1976 a,b) have shown that the conventional and defective vasectomy
did not affect the pituitary weights, its gonadotropin activity or urinary 17-ketosteroids.

Biochemical and electron spin resonance spectrometric investigations on the metabolism of the testis and epididymis of normal and vasectomized albino rats revealed that the alteration in the physiology of these organs following vasectomy were restored to normal level by extraneous feeding of ascorbic acid to the vasectomized rats. The data suggested that the beneficial effects of ascorbic acid and its mechanism of action have important implications in the prophylactic treatment during and following vasectomy (Chinoy et al., 1978 a). The metabolic significance of ascorbic acid turnover in rats, guinea pigs and human beings for maintaining the reproductive functions via the involvement of its free radical, monodehydroascorbic acid (MDHA) and the ascorbic acid charge transfer complex formation (AA-MM) have been reported from our laboratory (Chinoy and Sheth, 1977 c; Chinoy and Buch, 1977 b; Seethalakshmi and Chinoy, 1978; Chinoy et al., 1978 a-e).
The present investigation on ascorbic acid metabolism and some androgen dependent enzymic levels in the tissue components of testis and epididymis as well as testicular fluid and luminal plasma of epididymis from normal, vasectomized and vasectomized + ascorbic acid treated rats was an attempt to elucidate the beneficial role of ascorbic acid in vasectomized rats in the light of the recent data. This study was attempted in order to investigate the effects of vasectomy, if any, on the tissue components, tissue fluids of testis and epididymis separately as there is a paucity of data in this area.

2. STUDIES ON THE EFFECTS OF CYPROTERONE ACETATE ON RAT SPERMATOZOA

Cyproterone acetate (CA) has a potent antiandrogenic effect in human males and various laboratory animals (Gupta et al., 1974; Schenck and Neumann, 1978; Purvis et al., 1978; Neumann and Schenck, 1976; De La Torre et al., 1978; Van Wayjen and Van Den Ende, 1978; Sheth and Panse, 1978; Das et al., 1978; Govindarajulu and Umapathy, 1978; Moltz et al., 1978; Nag and Sarkar, 1976; Kalla and Bhasin, 1977; Arora-Dinakar et al., 1977;
Raman et al., 1977; Neri and Peets, 1975). It inhibits the action of androgens at both nuclear and cytosol receptor sites in target organs (Tveter, 1971; Brandes, 1974; Liao et al., 1974). If administered during the critical period of development, the differentiation of the male accessory sex organs is impaired (Steinbeck et al., 1970; Neumann, 1971/1972; Tymoczko and Liao, 1976).

Cyproterone acetate not only functions as an androgen antagonist but is also a potent progesteragen as it impairs the release of gonadotropins from pituitary (Hamada et al., 1963; Bloch and Davidson, 1967; Neumann et al., 1970; Neumann and Steinbeck, 1974; Neumann and Schenck, 1976; Eichner and Gupta, 1978). Cyproterone acetate also affects testicular functions and those of the male genital tract (Ogasa and Yokoki, 1970; Neumann, 1971/1972; Stern and Murphy, 1971; Hovatta, 1972; Heinert and Taubert, 1973; Neumann et al., 1976; Chinoy and Sheth, 1977b; Chinoy et al., 1978c,d, Chinoy and Chinoy, 1979). The synthesis of testosterone is reduced due to the inhibition of the enzyme systems (Grants and Stitch, 1973) and thereby a decrease in plasma levels of
androgens occur in CA treated animals and human volunteers (Brotherton and Bernard, 1974; Murray et al., 1975; Roy et al., 1976; Koch et al., 1976). That CA decreases androgen synthesis is substantiated by the recovery of several androgen sensitive parameters of the human semen in volunteers who were administered androgen along with CA (Roy et al., 1976).

Several studies on rodents and primates have shown that microquantities of CA released from subcutaneously implanted silastic capsules cause transient infertility in these animals (Prasad et al., 1971 / 1972, 1972 a, 1973 a, b; Prasad, 1973; Gupta et al., 1974; Bose et al., 1975; Rajalakshmi et al., 1976; Prasad and Rajalakshmi, 1977) by selectively inhibiting epididymal functions without altering the secretory activities of accessory reproductive organs and libido. Since the physiological integrity of the epididymis depends on the levels of circulating androgens as well as the normal flow of testicular fluid with sperm (Gustafsson, 1966; Rajalakshmi and Prasad, 1971), androgen deprivation by CA alters the internal milieu of the epididymis and
renders it hostile for the survival, motility and fertilizability of spermatozoa (Prasad et al., 1970; Rajalakshmi et al., 1971; Gupta et al., 1974; Arora et al., 1975; Chinoy et al., 1978 c, d; Roy et al., 1976; Koch et al., 1976). Therefore one of the most important antiandrogenic effects of CA is the alteration in the physiological milieu of epididymis via androgen deprivation. On the other hand, Schenck et al. (1975), Back and Schenton (1976) have failed to induce infertility by cyproterone acetate in rats, whereas, Buch (1976), Chinoy et al. (1978 c, d) and Chinoy and Chinoy (1979) reported pronounced inhibitory effects of CA on epididymal and vas deferens sperm motility and fertilizability.

The studies on CA in rats and guinea pigs have revealed its antianabolic effects with respect to the metabolism of reproductive organs. These antianabolic effects were transient and almost reversible by combined CA + ascorbic acid administration and CA withdrawal. However, fertility was not restored by these treatments. Ascorbic acid, therefore, has beneficial
effects in maintaining the metabolism of testis, epididymis and accessory organs in CA treated rats without interference with its antifertility effects. It has been suggested that the action of ascorbic acid is mediated by its enhanced free radical formation which potentiates the anabolic action of endogenous androgen (Chinoy et al., 1978 c, d). The reproductive tissue metabolism is therefore energized not only by high energy phosphate (~P) but also by the paramagnetic electron flow from the ascorbate free radical. On the basis of these results, it has been posulated that ascorbic acid and its mechanism of action may have important prophylactic implications in the treatment of human volunteers during and following CA treatment.

The present study was undertaken in order to investigate the effects of cyproterone acetate on the morphology of the rat epididymal spermatozoa, their density, motility pattern and fertilizability in animals treated with cyproterone acetate for different durations, those administered with cyproterone acetate and ascorbic acid and those of CA withdrawal. These studies were thought to be relevant in view of the
existing controversial data and since very little information is available on the morphology of the spermatozoa after CA treatment.

3. STUDIES ON CADMIUM CHLORIDE TREATMENT

Scrotal mammals possessing a complex vasculature are known to be more susceptible to cadmium chloride administration than non-scrotal animals (Chiquoine, 1964, Setty and Kar, 1964; Kar and Das, 1962; Erickson and Pincus, 1964; Kamboj and Kar, 1964; Kar and Kamboj, 1965; Kar et al., 1959; 1961; Lofts and Murton, 1967; Singh and Mathur, 1968). This effect is brought about since cadmium alters the permeability of testicular capillaries and the heat exchange mechanism of the testis (Gunn and Gould, 1970; Johnson and Vandemark, 1969). Cadmium chloride even in low doses has a potent antifertility action with detrimental effects on gonads and the accessory reproductive glands. It causes complete necrosis of the testis, inhibition of spermatogenesis, maturation of the spermatozoa and inactivation of several zinc-metallo enzymes and
those having -SH group (Kar et al., 1961; Kar and Das, 1962; Lohia and Dixit, 1974; Calvin et al., 1973; Thakkar et al., 1968; Roe et al., 1964; Seethalakshmi, 1976; Buchi, 1976; Timms et al., 1977; Chinoy and Sheth, 1977 d). It has also been reported that males are more susceptible to the effects of cadmium than females (Gunn et al., 1961; Kar et al., 1959; Gabbiani et al., 1967). The pituitary gonadotropic hormones and secretory activity of the accessory glands are altered as cadmium produces changes which are similar to those produced by castration (Gunn and Gould, 1970; Chinoy and Sheth, 1977 d). However, all the deleterious effects of cadmium chloride are dose, sex and species dependent and are reversible by administration of zinc (Gunn and Gould, 1970; Kar and Kamboj, 1965; Sarkar and Mondal, 1973). Similarly, the protective influence of ascorbic acid for the maintenance of the metabolism of the testis and epididymis, their spermatozoa and the accessory glands in cadmium chloride treated rats has been reported (Chinoy and Sheth, 1977 d; Chinoy et al., 1978 f). Patnaik (1971) also has reported enhanced depletion of ascorbic acid in the testis of rats injected with cadmium chloride.
The present study was therefore a further attempt to elucidate the metabolic turnover of ascorbic acid in epididymal and testicular spermatozoa, the epididymal sperm morphology of rats treated with cadmium chloride as well as cadmium chloride + ascorbic acid for different periods of time, as there is virtually no information on the repercussions of cadmium chloride treatment on sperm morphology.

4. STUDIES ON COPPER

A number of investigators have shown that copper inhibits the motility and metabolic activity of human spermatozoa in vitro and in vivo (Bernstein, 1958; Kesseru and Camacho-Ortega, 1972; Loewit, 1971; Oster, 1972; Ullmann and Hammerstein, 1972; White, 1955). The spermicidal activity of copper has been attributed to the binding of copper ions to the sulphhydryl groups of compounds present in the sperm (Mann, 1964; MacLeod, 1951; Oster and Salgo, 1977). The antifertility effect of copper wire devices or copper salts in silastic tubes implanted in the vas deferens and seminal vesicles of rodents and monkeys has also been reported (Gilmore et al., 1973; Ahsan et al., 1976; Khatun, 1978).
The intravasal copper appeared to be a promising technique for reversible control of fertility in the male rats and monkeys (Ahsan et al., 1976; Khatoon, 1978). The contraceptive effectiveness of the method is dependent on the proper placement of the device as well as on the surface area exposed to the vasal environment in the vasal lumen, while the reversibility depends on its careful removal with least damage to the vas tissue. The presence of the intravasal copper device seemed to produce no deleterious effects on the testis and accessory organs of the male reproductive system (Khatoon, 1978). However, Dixit and Jain (1977) have reported that copper wire implantation in the vas deferens of gerbils inflicted severe damage to the seminiferous tubules. The contraceptive effectiveness of the copper device has been attributed to either a direct toxic effect of copper on the spermatozoa present in the vas or to the involvement of copper ions in the binding of -SH groups of spermatozoa. The copper ions released from the intravasal copper device may also create a hostile environment for the sperm maturation in the epididymis.
and/or survival in the vas (Khatoon, 1978). Studies on the effects of intra-epididymal and intrascrotal copper device implantation in rats on their testis, epididymis and vas deferens in toto and accessory reproductive organs have revealed alterations in the metabolism of these tissues (Chinoy and Chinoy, unpublished data; Chinoy and Sharma, unpublished data).

Jecht and Bernstein (1973) have shown that copper ions released from the copper device have more effective toxic effects on the spermatozoa than copper salts. The possible displacement of zinc by copper in the spermatozoa may be important for affecting the sperm motility.

Copper ions injected into the laboratory animals produce testicular damage which is histologically similar to that produced by cadmium but to a lesser degree (Hoey, 1966). However, copper does not produce the severe necrosis which is characteristic of cadmium although there is some necrosis at the head of the epididymis by copper administration (Hoey, 1966).
The fact that copper is the most powerful catalyst for auto-oxidation of ascorbic acid and glutathione has implications in reproductive physiology. The cupric ion catalysed auto-oxidation of ascorbic acid generates free radicals which can bring about tissue damage (Oster and Salgo, 1977). This has a practical implication in terms of vitamin C deficiency for women taking oral contraceptives. The increased ceruloplasmin associated with exogenous estrogens is accompanied by reduced plasma levels of ascorbic acid. Therefore, a woman taking oral contraceptive must take about 500 mg vitamin C per day i.e., ten times the normal recommended daily level to compensate the increased loss (Oster and Salgo, 1977).

Vitamin C also has effect on copper metabolism. In copper deficient animals, the additional vitamin C makes the deficiency more severe. On the other hand, in copper toxicity the vitamin has a protective effect (Oster and Salgo, 1977; Prasad and Oberleas, 1976). Copper could also play an important role in reproduction via its destructive action on vitamin E. It acts synergistically with releasing hormones to cause the
release of gonadotropins (Suzuki et al., 1970; LaBella et al., 1973). Copper might also be involved in steroid metabolism, in the aromatization of steroids to estrogens, in the sulphonation of steroids and it inhibits the biosynthesis of prostaglandin E1 (Oster. and Salgo, 1977).

On the basis of the above mentioned data on the effect of copper in mammalian reproduction, especially of the male, the present study was undertaken to elucidate the effects of copper on sperm motility of rats in vitro as well as the effects of copper device implantation in the epididymis and scrotal region of rats and copper wire device + ascorbic acid feeding. The sperm density, motility, morphology, fertilizability, ascorbic acid metabolism and glutathione levels, in the spermatozoa, and the copper contents in the reproductive organs were investigated.

5. STUDIES ON PROSTAGLANDINS

The effects of prostaglandins on male reproductive functions are not adequately known. However, prostaglandins have been implicated in the process of ejaculation, sperm motility and morphology.
testicular and penile contractions and in steroidogenesis. Prostaglandins thus could have an influence on male potency and fertility (Pharris and Shaw, 1972; Karim, 1975; Ellis et al., 1975; Batta, 1975; Hinman, 1972; Shaw and Tillson, 1973; Davis et al., 1970; Hafs et al., 1974; Curtis-Prior, 1976; Kelly, 1978). The chronic subcutaneous injection of either PGE1 or PGE2 significantly decreased the testis and accessory gland weights and suppressed spermatogenesis and also decreased testosterone production in rats (Ericsson, 1972; Saksena et al., 1973). In mice, similar results were obtained by Abbatiello et al. (1975, 1976) wherein degeneration of spermatocytes and decrease in number of spermatids were noted. The other reports on the parenteral administration of PGF2a on various parameters of male reproductive functions were inconclusive (Bartke et al., 1973; Tso and Lacy, 1975). On the other hand, silastic discs containing 15 (s)-15 methyl PGF2a methyl ester (15-ME-PGF2a) implantation in the left side of the scrotum of rats caused an acute suppression of serum testosterone, LH and FSH together with decrease in the testicular and accessory gland weight, decreased or absent spermatogenic elements in
the testis and epididymis and hypertrophy of the interstitial cells (Kimball et al., 1978a). One week after the implantation of the silastic disc containing 1% of 15-ME-PGF2a reduced the potency and fertility which returned two weeks after implantation. The animals receiving implants of 2% PG containing discs were impotent the first week following implantation but the potency returned before fertility returned 11 weeks after implantation (Kimball et al., 1978b). Similarly, intrascrotal implantation of PGF2a in silastic tubes in fertile rabbits induced temporary sterility (Saksena et al., 1978a). A significant reduction in sperm number per ejaculate, testicular weight and the number of epididymal sperm accompanied the incidence of sterility which occurred 2-5 weeks after starting the treatment and persisted for 6-10 weeks. The libido and ejaculate volume were not affected. Saksena et al. (1978b) have also reported a significant reduction in the levels of serum testosterone, androstenedione, 5α-dihydrotestosterone and progesterone after 3-7 days of the intrascrotal insertion of silastic PVP tubes containing PGE2 and PGF2a in the male rats. Selective and age dependent changes of PGE2
in the epididymis and vas deferens of rats have been reported by Gerozissis and Dray (1977). Studies on effects of PGE1 and PGF2α on male reproduction of rats have revealed that both the prostaglandins exert antiandrogenic and antifertility effects as well as they antagonize the adrenergic response of the vas by blocking the α-receptors. The two prostaglandins also exhibit a growth promoting effect particularly in seminal vesicles and the ventral prostate (Chinoy and Chinoy, Unpublished observations; Chinoy and Sharma, Unpublished observations).

The present study was undertaken to investigate the effects of subcutaneous administration of low dose of PGE1 and PGF2α on the motility, density, fertilizability and morphology of rat epididymal spermatozoa. This study was attempted since no information is available in the literature on the morphology of spermatozoa after PG treatment although their motility and fertilizability have been reported to be affected.
Freeman and Coffey (1973 a, b) have developed a simple technique for achieving male sterility. This consists of inducing obstruction in the vas deferens by injecting sclerosing chemical agents through the skin of the scrotum directly into the vas. A similar technique was attempted by Setty et al., (1972) who have used a single injection into the vas deferens of camphor, potash alum or quinacrine. They reported diminished spermatozoa and occlusion of the distal portion of vas deferens lumen. On the other hand, obstruction of the vas deferens by injection of 95% ethanol was attempted by Freeman and Coffey (1973 a, b). They reported complete blockage of the vas deferens and the occurrence of sterility in large series of animals. The ejaculates were found to be completely free of spermatozoa following treatment which also produced pathological changes in the vas. Similarly, Raman et al. (1976) have reported changes in the histopathology of testis and epididymis of rats 3 and 6 months after vas occlusion either with ethanol-urea mixture or urea alone. A gradual decrease in the
fertility of rats was observed. The epididymis showed tubules with sperm granuloma and the testis had empty tubules with Leydig cell hyperplasia in the urea-ethanol treated animals. Dixit et al. (1976) have also studied the effects of vas occlusion by a single injection of potassium permanganate directly into the vas deferens of gerbils. These investigators did not observe any pathological changes in the testis and epididymis following injection into the vas deferens for a period of 3 weeks. The seminiferous tubules and Leydig cell functions were normal. The lumen of the epididymis showed the presence of normal spermatozoa. However, sperm granuloma occurred at the site of injection. The authors concluded that although vas occlusion was complete and the animals were sterile, but potassium permanganate treatment did not alter the histology and biochemistry of the reproductive organs of male gerbils. Dixit (1976) has reported that a single injection of α-chlorohydrin and/or cadmium chloride into the vas deferens of dogs produced an effective block in the lumen, caused severe degenerative changes in the testis and altered its biochemical composition. On the other hand, Poddar et al. (1975) used intravasal nylon thread in rats and observed no significant change
in the weight of testis, epididymis and accessory glands nor in the alkaline phosphatase activity of ventral prostate, but epididymal sialic acid was significantly reduced together with a marked reduction in the motility of spermatozoa in the distal vas. The occurrence of sperm granuloma and histological changes in the vas deferens of rats treated with alcohol and ascorbic acid separately have also been observed (Chinoy and Chinoy, unpublished observations). All the above mentioned studies have not considered the effects of the sclerosing chemical agents on the morphology and metabolism of the testicular and epididymal spermatozoa, specially of the latter. Hence the present study was undertaken to investigate epididymal sperm counts, motility, fertilizability, activity of some androgen dependent enzymes and the ascorbate turnover pattern of these spermatozoa in rats treated with ethanol as well as ethanol + ascorbic acid.
PART III

STUDIES ON NUTRITIONAL DEFICIENCIES AND THEIR EFFECTS ON MALE REPRODUCTIVE FUNCTION OF RATS AND GUINEA PIGS

1. EFFECTS OF PROTEIN FREE DIET IN RATS

Dietary imbalance such as inanition and protein malnutrition tend to affect the male reproductive functions in numerous ways, the most frequent being suppression of endocrine activity of the testis and consequently arrest of growth and diminished secretory activity of the male sex accessory glands. Deleterious influences are also manifested in the pituitary gonadal axis with atrophy of the pituitary gland, decreased gonadotrophin production and subsequently reduced androgen synthesis by testis as is manifested by less excretion of 17 ketosteroids, reduced testosterone/androgen ratio and reduction in the activity of \( \Delta^5 \) 3 \( \beta \)-hydroxy steroid dehydrogenase in the Leydig cells (Leathem, 1970; Mann, 1974). Another important factor which contributes to the malfunctioning of accessory glands in undernourished males is the decreased responsiveness of these glands to
testosterone (Platt et al., 1964; Leathem, 1970; Mann, 1974), resulting in their atrophy and decline in their secretory functions (Keys et al., 1950). A differential effect of malnutrition is manifested by different organs (Deo and Mathur, 1974), and it is known that male accessory reproductive glands are more sensitive than the testis. Chinoy and Sheth (1977 e) reported that the secretory function of seminal vesicle, coagulating gland and the prostate are more affected than other glands, indicating a decreased androgenicity of the testis together with a greater mobilization of the bound vitamin C for the maintenance of accessory gland function.

Alteration in endocrine function in protein calorie malnutrition has been reported by Pimstone (1976). Similarly, serum progesterone levels in pregnant rats, fed a protein free diet showed a significant decrease on day 11 of pregnancy and which remained low resulting in abortion (Giannina, and Leathem, 1974). A fall in the mean body weight in rats maintained on a protein deficient diet has been observed by Lal et al. (1975). The lesions of experimental protein deficiency
induced in rhesus monkeys were found to be completely reversible (Jha, 1977). However, chromosomal abnormalities in children suffering from protein calorie malnutrition was shown by Gupta et al. (1977).

Protein anabolic levels are higher in the tissues of young growing animals and the body is more dependent on dietary protein levels and quality than in adult animals. The amount of semen and sperm produced by the adult sheep have been related to dietary levels of protein. But numerous investigations on mature bulls have failed to prove that increased proteins improve the semen quality (Leathem, 1970). The weight of adult rat testis, spermatogenesis or the protein concentration were not affected by the removal of protein from the diet for 30 days. A prolonged protein depletion is necessary before the testis exhibits a loss in size. Protein malnutrition brought about by feeding a poor protein such as maize or gelatin decreased sperm motility and increased the number of abnormal sperms (Leathem, 1970).
The present study was undertaken in order to investigate the effects of 8 and 15 days of feeding a protein free diet on the metabolism, count, motility pattern, morphology and fertilizability of spermatozoa from the epididymis of protein deficient rats. The testicular spermatozoa of deficient animals were also studied.

2. EFFECTS OF VITAMIN C DEFICIENCY IN GUINEA PIGS

Guinea pigs, human beings and other primates are unable to synthesize ascorbic acid and they have to depend on dietary source (Burns et al., 1956; Hassan and Lehninger, 1956; Chatterjee et al., 1960). Ascorbic acid is known to participate in several biosynthetic reactions including steroidogenesis in gonads and adrenal via the formation of its free radical monodehydroascorbic acid (Fisher, 1962; Guchhait et al., 1963; Chinoy, 1969 a, 1970 a, b; 1972 a, b; 1973; 1978; Biswas, 1967, 1969; Biswas and Deb, 1970; Carballeiva et al., 1974; Sheth, 1976; Seethalakshmi, 1976; Buch, 1976; Parmar, 1978, Datta and Sanyal, 1978) and activates several
enzymes (Harrer and King, 1941; Sebrell and Harris, 1967; Kutsky, 1973). Metabolically active tissues contain higher turnover of ascorbic acid which has been correlated with the ability of the tissues to withstand stress conditions (Selye, 1950; Sheth, 1976; Buch, 1976; Parmar, 1978). The male guinea pigs are known to succumb to the effect of ascorbic acid deficiency sooner than females (Harman, 1950; Odumosu and Wilson, 1973), since vitamin C deficiency is known to be accompanied by atrophy of the testis, spermatogenic arrest, decreased androgen synthesis from cholesterol, a decline in succinate dehydrogenase and phosphatases together with an increase in lipids (Harman, 1950; Belavady, and Banerjee, 1954; Biswas, 1967; Chatterjee, 1967). Seethalakshmi and Chinoy (1976) reported a decrease in most of the androgen dependent parameters particularly in the cauda epididymis of vitamin C deficient guinea pigs and suggested that the internal milieu of epididymis was altered corresponding with the probable alteration in the levels of circulating androgen and testicular steroidogenesis, in conformity with the observations of others (Belavady and Banerjee, 1954; Banerjee et al., 1972). These authors
further reported that mobilization of stored ascorbic acid occurred from both the testis and cauda epididymis of vitamin C deficient guinea pigs. The ascorbic acid turnover was greater in the epididymis concurrent with an increased storage of the bound vitamin C therein. Buch (1976) reported decreased sperm motility, density, reduction in some androgen sensitive parameters and in bound ascorbic acid from epididymal spermatozoa of vitamin C deficient guinea pigs. Alterations in the content of nucleic acids and proteins also occur under acute ascorbic acid deficiency (Terroine, 1965). On the other hand, presence of motile spermatozoa and no specific degeneration of testis were observed in scorbutic guinea pigs (Mason, 1939; Goettsch, 1940).

In the light of these data, an investigation was undertaken to study the effects of induced vitamin C deficiency on sperm counts, motility, morphology, fertilizability and ascorbic acid turnover in guinea pigs.